The Pharmaceutical and Chemical Journal, 2019, 6(2):56-61

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Chemical Composition and antimicrobial activity of the Hexane Fraction of Sudanese *Acacia mellifera* (Fabaceae) Seeds

Abdel Karim M[1], Ezdehar A[2], Weam A K[3]

[1]Sudan University of Science and Technology, Faculty of Science, Sudan
[2]Jazan University, Samtah University College, Saudi Arabia
[3]Omdurman Islamic University, Faculty of Pharmacy, Sudan

Abstract Many *Acacia* species find wide applications in traditional medicine. Data on the medicinally important *Acacia mellifera* is very scarce. This study was designed to investigate the constituents of *Acacia mellifera* hexane fraction and to screen its antimicrobial potential. The hexane fraction was investigated by GC-MS analysis. The gas chromatogram revealed the following order of abundance: Fatty acids comprised the major constituents (87.40 %) followed the steroidal alcohol gamma-ergostenol (7.26 %), the aldehyde tridecanedial (3.44 %), hydrocarbons (1.81 %) and some terpenes and sesquiterpenes (0.09 %). The hexane extract was evaluated for antimicrobial activity against five standard human pathogens. At a concentration of 100mg/ml, the hexane extract showed moderate activity against all test organisms. It also exhibited moderate activity at 50mg/ml against *Pseudomonas aeroginosa*, *Bacillus subtilis* and the fungal species *Candida albicans*. Moderate activity against *Bacillus subtilis* and *Candida albicans* was also observed at 25mg/ml.

Keywords *Acacia mellifera*, Hexane extract, GC-MS analysis, Antimicrobial activity

Introduction

The genus *Acacia* (Fabaceae) contains around 1350 species [1]. Some Acacia species are rich in bioactive secondary metabolites such as flavonoids which have been reported from different *Acacia* species [2] and many Acacia species find wide applications in traditional medicine [3]. For example *Acaia nilotica* is a pioneer species and considered as a source of gum, timber and fodder [4]. Various parts of *Acaia nilotica* are used in Sudanese system of medicine. Leaves are antipyretic, astringent, tonic and a remedy for dysentery and diarrhea [5-7]. Bark is used traditionally against leucorrhoea, piles and vaginitis [8]. The roots are used for wounds and leucorrhoea [9]. Pod is a remedy for cough, impotency and urino-genital disorder [10], while gum is used against diabetes [11]. *Acacia nilotica* is also used traditionally in Sudan as a remedy for malaria, sore throat and intestinal worms [12-15].

Acacia seyal- is considered as a safe dietary fiber by the United States Food and Drug Administration and its therapeutic uses were extensively examined in animal models [16,17]. The antioxidant activity of the medicinally important *Acacia auricoliformis* has been documented [7] and some *Acacia* species were claimed to exhibit potent antimicrobial activity [18].

Data on the medicinally important *Acacia mellifera* is very scarce. However, a methylated dihydrochalcone has been reported from *Acacia mellifera* bark [19].

In continuation of our interest in the bioactive constituents of *Acacia* species , this study was designed to investigate the chemical constituents of *Acacia mellifera* oil and to evaluate its antimicrobial potential.

Materials

Plant material

Acacia mellifera seeds were collected from Damazin (Sudan) and authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum-Sudan.

Instruments

A Shimadzo GC-MS-QP2010 Ultra instrument (column: 30m, length; 0.25mm, diameter; 0.25 μm, thickness) was used for GC-MS analysis**.**

Test organisms

Acacia mellifera oil was assessed for antibacterial and antifungal activities using: *Bacillus subtilis*, *Staphylococcus aureus* (G+ve), *Pseudomonas aeroginosa*, *Escherichia coli* (G-ve) and the fungal species *Candida albicans* .

Methods

Extraction of oil

Three hundred grams of powdered seeds were macerated with n-hexane. The solvent was evaporated *in vacuo* to give the oil. For GC-MS analysis the oil was esterified by methanolic sodium hydroxide and methanolic sulphuric acid.

GC-MS Analysis

Acacia mellifera hexane fraction was analyzed by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column. The oil was dissolved in dichloromethane and directly injected into the capillary column under the following chromatographic conditions: injection volume: 1μl; injection temperature: 250 ^{o}C; flow rate of helium: 1.2ml/min.; injection mode: split (ratio 300); the oven was raised from 35 ^{o}C (hold for 3 min.) to 240 ^{o}C at a rate of 5^{o} C/min., then at a rate of 3 ^{o}C/min., raised to 280 ^{o}C, hold for 3 min.; interface temperature was 250 ^{o}C; ion source temperature: 200 ^{o}C ; start time: 4 min. ; end time: 62.23 min.; mass scan range: m/z 10-600.

Antimicrobial Susceptibility

Aliquots (1ml) of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^{8}-10^{9} colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

Fungal cultures were maintained on sabouraud dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Plate agar diffusion assay was used to screen the antibacterial activity of the oil. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes, the agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4). Separate Petri dishes were designed for standard antibacterial chemotherapeutic, (ampicillin and gentamicin).

The agar discs were removed, alternate cup were filled with 0.1 ml of test sample using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured in duplicates and averaged.

The above procedure was used for antifungal activity, but Sabouraud dextrose agar was used.

Results and Discussion

The investigation of the constituents of *Acacia mellifera* hexane fraction was accomplished by GC-MS analysis using a Shimadzo GC-MS-QP2010 Ultra instrument. Gas chromatogram revealed the presence of 38 components (their abundance is depicted in Table 1 and Fig. 1). Fatty acids comprised the major constituents (87.40%) followed the steroidal alcohol gamma-ergostenol (7.26%), the aldehyde tridecanedial (3.44%), hydrocarbons (1.81%) and some terpenes and sesquiterpenes (0.09%).

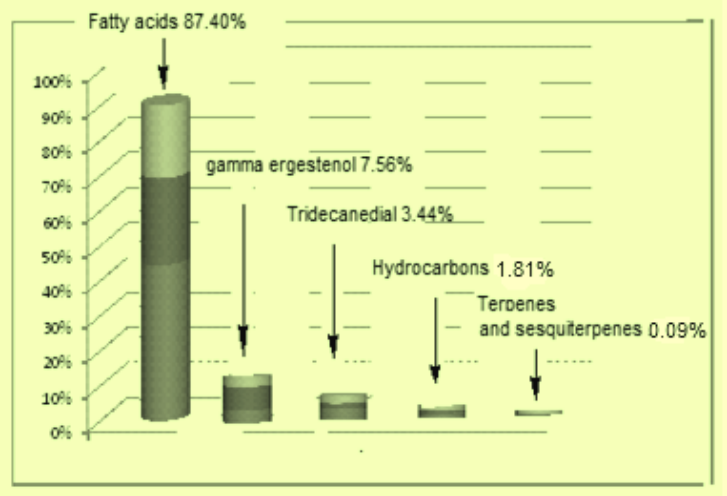

Figure 1: Abundance of constituents of the hexane fraction

Table 1: Constituents of the hexane fraction

No.	R. Time	Area%	Name
1	4.583	0.01	6-Heptenoic acid methyl ester
2	7.152	0.04	A-Terpineol
3	7.243	0.01	Cyclohexanol-1-methyl-4-methylethyl
4	11.031	0.01	10,12-Docasadiyndioc acid
5	11.166	0.02	Beta-curcumene
6	11.268	0.01	Alpha-Farnesene
7	11.332	0.01	Isocayophillene
8	11.425	0.03	Dodecanoic acid methyl ester
9	11.538	0.01	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-
10	13.434	0.01	Apiol
11	13.474	0.01	Cis-5-Dodecenoic acid methyl ester
12	13.749	0.31	Methyl Tetradecanoate
13	14.561	0.02	5-Octadecenoic acid methyl ester
14	14.828	0.05	Pentadecanoic acid methyl ester
15	15.562	0.02	n-Propyl-9,12-hexadecadienoate
16	15.621	0.13	7-Hexadecenoic acid methyl ester
17	12.667	1.64	9-Hexadecenoic acid methyl ester
18	15.760	0.08	11-Hexadecenoic acid methyl ester
19	15.882	12.92	Hexadecenoic acid methyl ester

20	16.630	0.28	Cis-10-Heptadecenoic acid methyl ester
21	16.840	0.51	Heptadecanoic acid methyl ester
22	17.544	17.55	9,12-Ocadecadienoic acid methyl ester
23	17.623	12.45	9-Otcadecenoic acid methyl ester
24	17.809	11.23	Methyl stearate
25	18.676	0.10	Nonadecanoic acid methyl ester
26	19.182	3.44	Tridecanedial
27	19.305	3.25	Oxiraneoctanoic acid,3-octyl methyl ester
28	19.340	1.37	11-Eicosenoic acid methyl ester
29	19.549	6.54	Eicosanoic acid methyl ester
30	19.604	0.88	PGH1 methyl ester
31	19.711	1.13	Methyl 15-hydroxy-9,12- ocadecadienoic acid methyl ester
32	20.365	0.50	Heneicosanoic acid methyl ester
33	21.070	5.55	Fumaric acid, 3-heptyl nonyl ester
34	21.174	6.51	Docosanoic acid methyl ester
35	21.515	1.51	1-Nonadecene
36	21.926	0.76	Tricosanoic acid methyl ester
37	22.669	3.58	Tetracosanoic acid methyl ester
38	23.811	7.56	ɞ-Ergostenol
		100%	

The major constituents of the hexane fraction are briefly discussed below:

i)- 9,12-Ocadecadienoic acid methyl ester(17.55%)

The mass spectrum of 9, 12-octadecadienoic acid methyl ester is depicted in Fig.2. The signal which was observed at m/z294(R.T. 17.544) is due to M+[$C_{19}H_{34}O_2$]+, while the signal at m/z263 corresponds to loss of a methoxyl.

ii)- Hexadecanoic acid methyl ester(12.92%)

The mass spectrum of hexadecanoic acid, methyl ester is displayed in Fig.3. The peak at m/z 270 (R.T. 15.882) accounts for M^+ [$C_{17}H_{34}O_2$] $^+$. The signal at m/z 239 is due to loss of methoxyl.

iii)- 9-Octadecenoic acid methyl ester(12.45%)

The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig. 4. The signal at m/z296 (RT, 17.623) is due to the molecular ion M+[C19H36O2]+. The peak at m/z266 is due to loss of a methoxyl.

iv)- Methyl stearate(11.23%)

The mass spectrum of methyl stearate is shown in Fig. 5. The peak at m/z 298 (R.T.17.809) is due to M^+ [$C_{19}H_{38}O_2$]$^+$, while the signal at m/z 267 correspond to loss of a methoxyl.

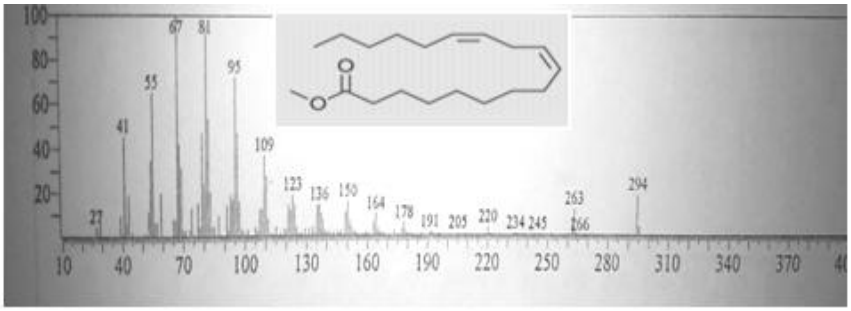

Figure 2: mass spectrum of 9,12-octadecadienoic acid methyl ester

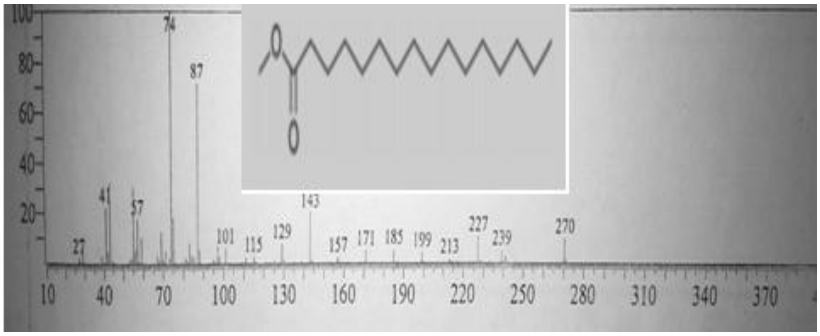

Figure 3: mass spectrum of hexadecanoic acid, methyl ester

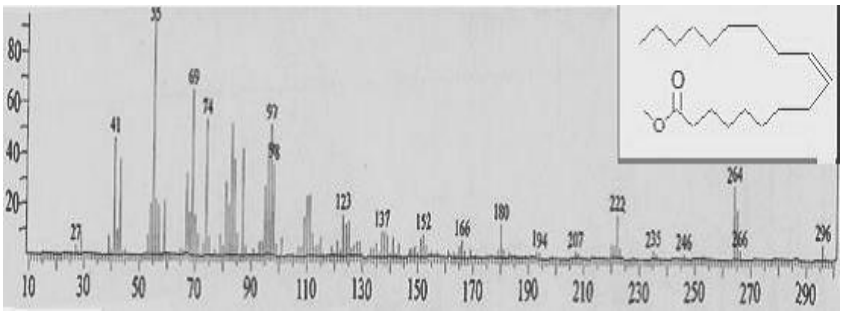

Figure 4: Mass spectrum of 9-octadecenoic acid methyl ester

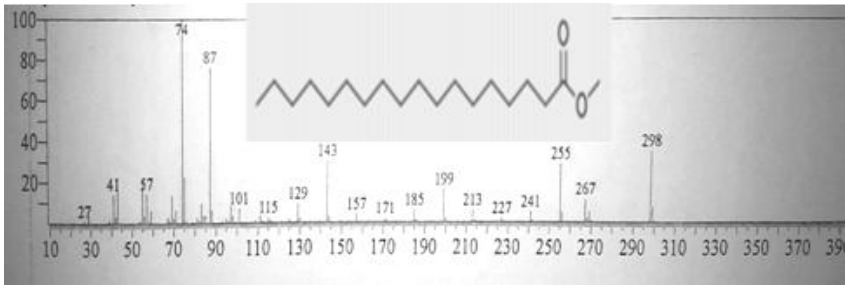

Figure 5: mass spectrum of methyl stearate

Antimicrobial activity

In the cup plate diffusion assay, *Acacia mellifera* hexane fraction was assessed for antimicrobial potential using five standard human pathogens: *Bacillus subtilis, Staphylococcus aureus* (G+ve), *Pseudomonas aeroginosa*, *Escherichia coli* (G-ve) and the fungal species *Candida albicans*. The inhibition zones are shown in Table 2.

At a concentration of 100mg/ml, the hexane extract showed moderate activity against all test organisms. It also exhibited moderate activity at 50mg/ml against *Pseudomonas aeroginosa*, *Bacillus subtilis* and the fungal species *Candida albicans*. Moderate activity against *Bacillus subtilis* and *Candida albicans* was also observed at 25mg/ml. Ampicilin, gentamycen and clotrimazole were used as positive controls, while DMSO was used as negative control (Tables 2).

Table 2: Antibacterial activity of the hexane extract

Type	Conc. (mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	13	15	15	14	15
	50	12	14	12	13	14
	25	12	13	10	12	14
	12.5	10	12	10	--	--
	6.25	10	13	7	--	--

Ampicilin	40	30	15	--	--	--
Gentamycin	40	19	25	22	21	--
Clotrimazole	30	--	--	--	--	38

References

[1]. Seigler, D. S., *Biochemical Systematics and Ecology*, 31, 2003, 845.

[2]. Gaara A. H., Nassar M. I., Younis, M., Elmegeed, G. A., Mabry, T. J., Pare, P. W. *Latinoamer. Quim., 36*, 2008, 52.

[3]. Boulos, L. "Medicinal Plants of North Africa" Algonac, Michigan, P 115(1983).

[4]. Gupta RK. Resource survey of gummiferous acacias in western Rajasthan. *Tropical Ecology*. 1970; 11:148-161.

[5]. Nadkarni KM. The Indian Plants and Drugs. New Delhi: Shrishti Book Distributors2005; 4:5.

[6]. Narayan DP, Kumar U. "Agro's Dictionary of Medicinal Plants". Jodhpur; Updesh Purohit for Agrobios. 2005,P. 352- 353

[7]. Saini ML. Comparative Pharmacognostical and antimicrobial studies of Acacia species (Mimosaceae). *Journal of Medicinal Plants Research*. 2008; 2(12):378- 386.

[8]. Kritikar KR, Basu BD. "Indian Medicinal plants with illustrations" (2nd Ed.), Uttaranchal Oriental Press. 2003.

[9]. Gilani AH, Shaheen F, Zaman M, Janbaz KH, Shah BH, Akhtar MS. Studies on antihypertensive and antispasmodic activities of methanol extract of Acacia nilotica pods. *Phytotherapy Research*. 1999; 13:665-669

[10]. Mohanty RV, Padhy SN, Das SK. Traditional phytotherapy for diarrhoeal disease in Ganjan and phulbani District of South Orissa, India. *Ethnobotany.* 1996; 8:60-65.

[11]. Chopra RN, Nayar SL, Chopra IC. "Glossary of Indian Medicinal Plants" (6th Ed.). New Delhi NISCIR, 2002, 2.

[12]. Shetty K A B, *Indian Farming*, 26(11), 1977, 82.

[13]. Joshi P, Ethnomedicine of Tribal Rajasthan - An over view; In: Pushpangadan (Eds.)," Gilmpses of Indian Ethnopharmacology", TBGRI, Thiruvunanthapura, India, 1994; 147-162.

[14]. Jain A, Katewa S S, Galav P K and Sharma P, *Indian J Ethnopharmacol.*, 2005, 102(2) , 143.

[15]. Kubmarawa D, Ajoku G A, Enwerem N M and Okorie D A, *Afr. J. Biotechnol*., 2007 6(14), 1690.

[16]. Sahni M, "Important Trees of the Northern Sudan", Khartoum University Press, 1968, 40-63.

[17]. Rasha, B., Tarig, H., Khalifa, E., Rehab, M., Florian, L. and Amal, M., *Nutr. J.*, 2012, 11, 111.

[18]. Almagboul A. Z., Bashir A. K., Saleh A. K., Farouk A., Khalid S. A., *Fitoterapia*, 59, 1988, 57.

[19]. Tohami, E., Ph.D. Thesis, Sudan University of Science and Technology, 2016.